

Penicillin Resistance of Nonvaccine Type *Pneumococcus* before and after PCV13 Introduction, United States

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Introduction of 13-valent pneumococcal conjugate vaccine in the United States was not associated with a significant change in prevalence of penicillin resistance in nonvaccine type serotypes because of the variable success of highly resistant serotypes. Differences in regional serotype distribution and serotype-specific resistance contributed to geographic heterogeneity of penicillin resistance.

Streptococcus pneumoniae (pneumococcus) causes a range of debilitating and potentially life-threatening infections, such as pneumonia, meningitis, and septicemia. To reduce illness and death caused by pneumococcal diseases, a 7-valent pneumococcal conjugate vaccine (PCV7) was introduced in 2000 and targeted serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F. However, although vaccine type serotypes declined in frequency after PCV7 introduction (1,2), an increasing frequency of nonvaccine type (NVT) serotypes in samples from carriage and invasive disease was observed in subsequent years (2,3). Known as serotype replacement, this population-level change in serotype distribution, which most often involves preexisting clones and serotypes that were already in circulation before vaccine implementation (4), can reduce the benefits of vaccination (5). To address the rise in invasive pneumococcal disease associated with NVT serotypes, a second-generation conjugate vaccine was implemented in 2010 (PCV13), targeting the 7 serotypes targeted by PCV7 plus 6 additional serotypes: 1, 3, 5, 6A, 7F, and 19A (6).

The prevalence of penicillin-resistant pneumococcus strains varies considerably between states (7,8). Such variation might be caused by differences in serotype distribution (such that some locations have a higher prevalence of strains that are generally more resistant) or higher-than-average levels of resistance within serotypes. Before the introduction of PCV7, regional variations in the prevalence of antibiotic resistance were considered to be caused by regional

differences in antibiotic use, leading to differences in the intensity of selective pressure acting on the bacterial population (9). The variation in the proportion of resistant isolates within individual serotypes in the United States was thought to be a reflection of this regional difference in antibiotic use and was identified as the major factor in driving geographic variation of penicillin resistance (7). However, post-PCV7, this factor played a diminishing role in explaining geographic heterogeneity in penicillin resistance, with variation in serotype distribution between sites being of increasing importance (8). Understanding the underlying causes of the geographic heterogeneity of penicillin resistance and the role of selective pressure provides important insights on the long-term dynamics of penicillin resistance in the United States.

The Study

To analyze NVT penicillin-nonsusceptible pneumococcus (PNSP) detected in patients with invasive pneumococcal disease, we used data from the Active Bacterial Core surveillance (ABCs) system, a population- and laboratory-based collaborative system between the Centers for Disease Control and Prevention and state health departments and academic institutions in 10 states (California, Colorado, Connecticut, Georgia, Maryland, Minnesota, New Mexico, New York, Oregon, and Tennessee). We considered PNSP non-PCV13 serotypes detected in patients in all age groups from 2009 (pre-PCV13, $n = 285$ patients) through 2012 (post-PCV13, 339 patients). Nonsusceptibility was based on the meningitis breakpoint (MIC ≥ 0.12 $\mu\text{g/mL}$), as recommended by the Clinical and Laboratory Standards Institute (10). Serotypes 15B, 15C, and 15B/C were grouped together as 15BC because of the reported reversible switching between the 2 serotypes, which makes the precise differentiation of these serotypes difficult (11).

To determine whether geographic differences in the proportions of PNSP were consistent across serotypes, we calculated the proportions of PNSP for each of the 7 most common NVT serotypes (15A, 15BC, 16F, 23A, 23B, 33F, and 35B) across the 10 sites for 2009 and 2012. We found that serotypes with the highest proportions of PNSP in 2012 already had high resistance in 2009 (Figure 1). We calculated the Spearman correlation coefficient between the proportion of PNSP for each pair of serotypes across states in 2009 (range -0.09 to 0.66) and 2012 (range 0.30 – 0.79)

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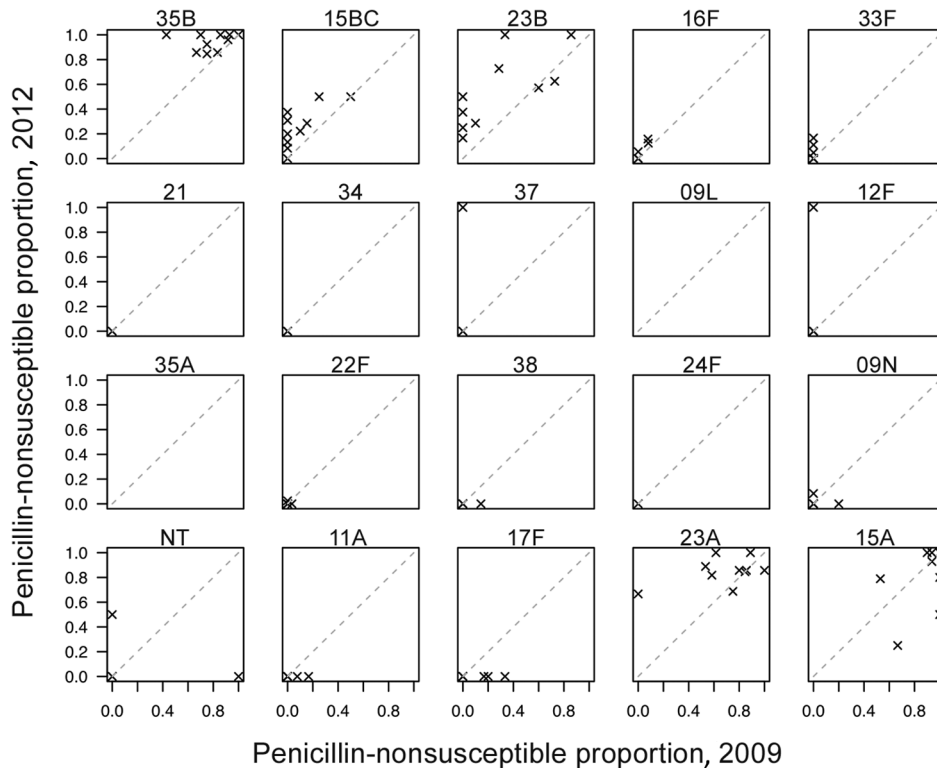


Figure 1. Comparison of proportion of nonvaccine type serotypes with penicillin resistance, by serotype, United States, 2009 and 2012. Based on Active Bacterial Core surveillance system data from 10 US states. The dashed diagonal line represents no change.

(online Technical Appendix, <https://wwwnc.cdc.gov/EID/article/23/6/16-1331-Techapp1.pdf>). We found significant overall correlation in 2009 and 2012 ($p < 0.001$ for both years), indicating that sites with high proportions of PNSP in 1 serotype typically will also have high proportions of PNSP in other serotypes. This finding suggests that differences in selection pressure account for the geographic variation in the proportions of PNSP.

We next implemented a standardized regression approach, used previously to analyze the pneumococcal-resistance patterns pre-PCV7 (7) and post-PCV7 (8) (online Technical Appendix). To investigate the source of geographic variation in the proportion of PNSP, we tested the hypotheses that either geographic heterogeneity in serotype distribution (Std1), or serotype-specific differences in penicillin resistance (Std2) were responsible for the observed variation. These effects were quantified by regressing crude versus standardized prevalence of penicillin resistance (Figure 2; online Technical Appendix), by which a regression slope of 1 would indicate that the factor considered had zero effect. By using 2009 data, we found that regression slopes for Std1 (0.445, 95% CI –0.083 to 0.972) and Std2 (0.463, 95% CI –0.013 to 0.939) indicate that both factors played a similar intermediate role in generating this geographic variation in penicillin resistance, with neither 95% CI containing 1. In 2012, the regression coefficient for Std2 was higher (0.634, 95% CI 0.14–1.128), whereas the coefficient for Std1 decreased (0.367, 95% CI –0.025 to 0.758). Although these

changes are not statistically significant relative to 2009, they might suggest shifting contributions to the observed variation in proportions of PNSP after the introduction of the PCV13 vaccine in 2010, with geographic differences in serotype distribution having an increased role and differences in serotype-specific PNSP becoming less important.

Finally, we sought to quantify the rate of change in penicillin resistance during 2009–2012 in each state. We documented the proportion of PNSP by state for the pre- and post-PCV13 periods (online Technical Appendix Table 4). No significant change in state-level resistance was observed. New Mexico, Maryland, and Georgia saw the highest increases in the proportion of PNSP during 2009–2012, whereas a slight decline was observed for Colorado, New York, and Connecticut. Although the distribution of serotypes might greatly fluctuate among geographic regions immediately after vaccine introduction, the overall proportions of NVT serotypes with penicillin resistance across the country might not vary significantly between the pre- and post-vaccine periods. Of potential importance are the small increases in the proportions of PNSP serotypes not included in either vaccine that were observed between the implementation of PCV7 and PCV13 (12), which might lay the foundation for changes post-PCV13.

Conclusions

The marked variation in the proportion of penicillin resistance among states highlights the potential of local

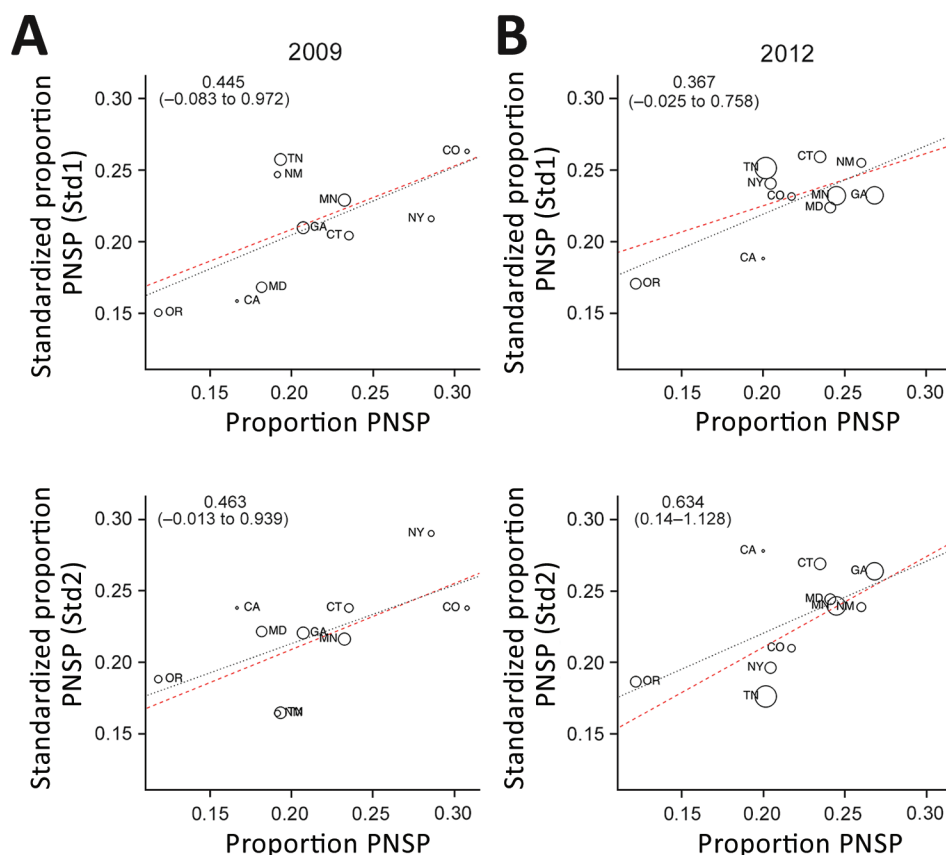


Figure 2. Crude versus standardized proportions of nonvaccine type serotypes with penicillin nonsusceptibility, by state, United States, 2009 and 2012, based on Active Bacterial Core surveillance system data from 10 US states. Std1 denotes standardization for geographic heterogeneity in serotype distribution. Std2 denotes standardization for serotype-specific differences in resistance. Regression slopes with 95% CIs are indicated in the upper left corner of each panel. Larger circles represent states with a greater number of penicillin-resistant samples. Dashed lines represent the inverse-variance weighted (red) and unweighted (gray) regression slopes. PNSP, penicillin-nonsusceptible pneumococcus.

selective pressures to favor certain serotypes and resistant strains within each serotype to increase in frequency as the population returns to equilibrium (13). Previous studies have already shown significant regional differences in antibiotic use and vaccination coverage across the United States (14,15). Regional rates of patient adherence to treatment regimens will also influence variations in resistance. A combination of these factors, which will likely vary between and within regions, would greatly affect proportions of resistance across the country.

In our study, we observed that the dynamics of penicillin resistance continue to shift in the wake of vaccine introduction. Our postvaccine observations were recorded shortly after the introduction of the vaccine; additional observations would be valuable to determine the stability of the postvaccine dynamics and any potential importance of the temporal changes we observed to factors contributing to variation in resistance levels. Further long-term nationwide surveillance of serotype dynamics is required to assess the multiple ecologic factors that influence antibiotic resistance in the pneumococcus in the conjugate vaccine era.

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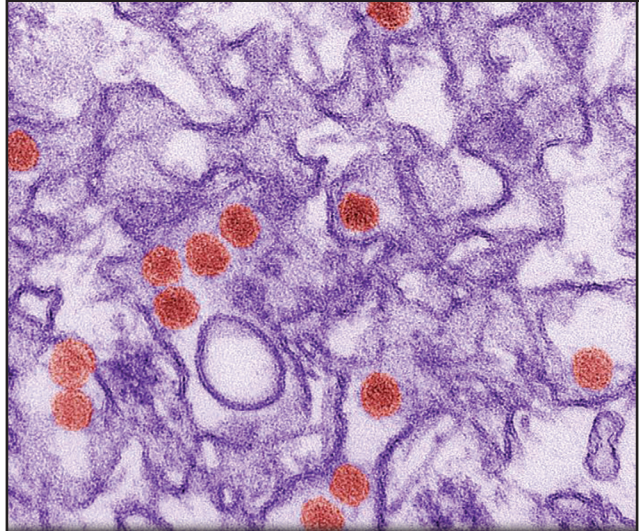
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Zika virus (ZIKV), a mosquito-transmitted flavivirus, has been isolated from sentinel monkeys, mosquitoes, and sick persons in Africa and Southeast Asia. Serologic surveys indicate that ZIKV infections can be relatively common among persons in southeastern Senegal and other areas of Africa, but that ZIKV-associated disease may be underreported or misdiagnosed. In 2007, a large outbreak of ZIKV infection occurred on Yap Island in the southwestern Pacific that infected ~70% of the island's inhabitants, which highlighted this virus as an emerging pathogen. The purpose of this study was to investigate and report 3 unusual cases of arboviral disease that occurred in Colorado in 2008

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Penicillin Resistance of Nonvaccine Type Pneumococcus before and after PCV13 Introduction, United States

Technical Appendix

Methods

We implemented the standardization method used by McCormick and colleagues to analyze the pre-PCV7 pneumococcus dataset from the ABCs system (1). The same method was also used by Link-Gelles and colleagues to compare the pre-PCV7 and post-PCV7 pneumococcal populations (2). In both studies, standardized proportions were calculated to reflect either the differences in serotype composition or the differences in proportions of resistance against penicillin and erythromycin within individual serotypes, in their contribution to the prevalence of PNSP at each sample site.

The observed prevalence of resistance may vary by state for several reasons. Antibiotic usage across the ten states is highly non-uniform, and resistance to penicillin and other antibiotics has been shown to be significantly associated with prescription rates (3). If there were geographic heterogeneity in selective pressure due to varying antibiotic usage, one would expect correlation in the prevalence of penicillin resistance between serotypes (2). To test this, we calculated the Spearman's correlation coefficient c_{ij} between the proportion of penicillin resistance for each pair of serotypes i and j across states in both 2009 and 2012. We considered the seven most common NVT serotypes (15A, 15BC, 16F, 23A, 23B, 33F, 35B). While other NVTs were also present, none were significant contributors to the PNSP population at any study site. Taking the sum of these coefficients

$$C = \sum_{i,j} c_{ij}$$

as a test statistic, we could assess the significance of site-specific selective pressure using a permutation test to determine whether correlation was greater than would be expected at random.

We next investigated to what extent observed differences in penicillin resistance prevalence could be attributed to:

1. Spatial heterogeneity in serotype distributions (Std1),
2. Serotype-specific prevalence of penicillin resistance (Std2).

We standardized the observed prevalence of penicillin resistance in each state, adjusting for each of the two factors Std1 and Std2 (1,2). We performed an inverse-variance weighted linear regression between the crude observations and the standardized values. If the observed and standardized values are identical (linear regression coefficient 1), then the factor under consideration has no effect on the observation. Conversely, if the observed and standardized values have no relationship (linear regression coefficient 0), then the factor under consideration is completely responsible for the observations. We repeated this analysis for observations in 2009 (post-PCV13) and 2012 (post-PCV13). Finally, we calculated the rate of change for prevalence of penicillin resistance between 2009 and 2012 for each state under a logistic growth model.

Results

A total of 3,746 pneumococcal isolates of all serotypes were collected in 2009 and 2,740 in 2012, of which a total of 1,312 (35.02%) and 1,488 (54.31%) were NVTs respectively. The increase in proportion of IPD cases resulting from NVTs was significant ($p < 0.05$) in all but one state (California, $p = 0.057$), which might be attributable to the removal of VT in the population although serotype replacement may also be playing a role. The five most common NVTs collected in 2009 were 22F ($n = 249$; 19% of all NVTs), 33F ($n = 119$; 9%), 23A ($n = 114$; 9%), 16F ($n = 111$; 9%) and 15A ($n = 109$; 9%), while 2012 was dominated by serotypes 22F ($n = 333$; 22%), 09N ($n = 117$; 8%), 33F ($n = 115$; 8%), 35B ($n = 114$; 8%) and 15BC ($n = 108$; 7%). The proportions of NVTs as a total of sampled IPD cases differed significantly across states both in 2009 ($p = 0.04$) and 2012 ($p < 0.001$) (Technical Appendix Figure 1).

A total of 285 (22%) NVTs were penicillin resistant in 2009 and 339 (23%) in 2012. The NVTs exhibiting by far the highest proportions of penicillin resistance in the sample were 35B, 23A, 15A, 23B and 15BC (Technical Appendix Table 1). A significant increase (from 9% to 28%, $p = 0.01$) in the frequency of isolates carrying serotype 15BC was observed between 2009

and 2012, along with non-significant increases in 35B, 23A and 23B (Figure 1; Technical Appendix Table 1). The observed prevalence of PNSP varied considerably by serotype and between sample sites. In particular, serotypes 23A, 15A and 23B showed marked changes in the proportion of isolates that were susceptible while the great majority of serotypes show no increase (or decrease) between 2009 and 2012 (Technical Appendix Figures 2 and 3).

The introduction of serotype-specific pneumococcal vaccines can result in large fluctuations in the structure and dynamics of pneumococcal populations. Serotype replacement may partially offset the benefits of vaccination over the long term until a PCV targeting additional serotypes is implemented, as was observed with PCV7 (4). While no replacement disease has yet been reported following PCV13, the potential for increasing resistance in NVT is of great concern, particularly since serotype replacement has been reported in pneumococcal carriage (5–7). Previous studies of NVTs have reported that vaccines have altered the population structure of NVTs through serotype replacement (8–11). During the post-PCV7 period, there was an observed increase in PNSP among NVTs (12–14), but the underlying causes are not fully understood. In serotypes 15A, 23A, and 35B, penicillin nonsusceptibility shifted in the post-PCV7 era mainly as a consequence of shifts of clonal populations within individual serotypes that were comprised of distinct susceptible and nonsusceptible clonal complexes (13), but other factors might explain increased resistance in other serotypes. Understanding this is critical as it is anticipated that a complete or near complete replacement of the PCV13 serotypes in carriage is likely to occur once the population has regained equilibrium, as was observed in different parts of the United States after PCV7 implementation (5,15–17). While the antibiotic resistance profiles of the replacing NVTs from disease are known, as NVTs become more common over time greater exposure to antibiotics may produce selection for increased resistance. In this study, we found that while prevalence of PNSP remained largely constant following PCV13 introduction, there was much geographic heterogeneity and variation by serotype, which should be monitored closely to detect emerging threats.

We implemented the standardization method used by McCormick and colleagues to analyze the pre-PCV7 pneumococcus dataset from the ABCs system (1). The same method was also used by Link-Gelles to compare the pre-PCV7 and post-PCV7 pneumococcal populations (2). In both studies, standardized proportions were calculated to reflect either the differences in serotype composition or the differences in proportions of resistance against penicillin and

erythromycin within individual serotypes, in their contribution to the prevalence of PNSP at each sample site. In contrast to the two previous studies in which the whole sampled pneumococcus population was analyzed and all serotypes were included, we standardized the geographic differences in penicillin resistance as a proportion of the NVT population only.

Two factors play an important role in producing the geographic variation in the proportion of penicillin resistance: differences in the serotype composition and differences in the proportions of penicillin resistance within individual serotypes, which is primarily due to selection of pre-existing resistant clones within serotype. Our results suggest that both have equally contributed to the observed variation before the introduction of PCV13, but serotype distribution played a slightly greater role post-vaccine. The contribution of serotype distribution is likely influenced by changes in the distribution of VT, such that antibiotic pressure is applied only to the serotypes currently circulating at any given point in time.

Our results were in contrast to a similar study conducted on the population before PCV7 use that showed geographic variation in penicillin resistance is attributable primarily to within-serotype proportions of penicillin resistance - and hence implicated differences in antibiotic use among sites (*1*). However, our results for 2009 were similar to those reported by Link-Gelles and colleagues for pneumococcus collected in the later years of post-PCV7 (2007–09) (*2*), indicating that both factors drive the geographic variation in penicillin resistance when the population is close to equilibrium.

Our standardization results for 2012 were also in direct contrast to those reported for the years immediately following PCV7 introduction (2002–06) (*2*). Although both factors remain important during these time periods, our study shows that serotype composition played a slightly greater role, while the previous study reported a greater contribution by the differences in the proportion of penicillin resistance within each serotype. Two reasons may explain the disparity between our results and those of Link-Gelles and colleagues (*2*). First, since our focus was only on NVTs, rather than both VT and NVT isolates as used in the previous study ($n = 6,929$), the small sampling size in our study naturally leads to increased uncertainty. Alternatively, short-term dynamics may be different between the two groups of serotypes. We can suggest that immediately following PCV implementation, there is a rapid and stochastic shuffling of different genotypes within the local population at each site as a result of the opening up of new niches

following the elimination of VTs, and which NVTs are involved at each site will depend on which happened to be present before vaccination. For the VTs remaining in the population post-vaccine, differences in the proportion of penicillin resistance within individual serotypes remain as the primary driving factor mainly because some of these VTs, despite the declining numbers, continue to be present in relatively higher frequencies when the population has still not yet reached equilibrium. This is the case for serotype 19A, which remains an important disease-causing and highly resistant serotype, even though it has declined in frequency during the early post-PCV13 period and NVTs are now primarily detected (11,18). Hence, the geographic variation in penicillin resistance of NVTs will therefore largely depend on which NVTs were present in the population before the vaccine was implemented, even if they occurred initially at very low frequencies.

One limitation of our study is that we did not consider the effects of serotype-specific differences in the proportion of penicillin resistance by age group (14). This variation may also be affected by differences in antibiotic pressure due to differences in antibiotic consumption between children and adults (19). While our study provides a quantifiable assessment of the relative contributions of serotype distribution and serotype-specific proportions of penicillin resistance in NVT, future investigation should focus on age-related differences in resistance to specifically identify the underlying causes of such variation.

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Technical Appendix Table 1. Changes in the proportions of penicillin resistance in the most abundant resistant serotypes between 2009 and 2012

Serotype	Proportion resistant, N resistance/N total (%)	
	2009	2012
35B	61/78 (78%)	108/114 (95%)
23A	84/114 (74%)	87/103 (85%)
15A	96/109 (88%)	69/83 (83%)
23B	21/63 (33%)	28/56 (50%)
15BC	7/76 (9%)	30/108 (28%)*
Others	15/872 (2%)	18/924 (2%)

* Significant increase (Fisher exact test).

Technical Appendix Table 2. Spearman correlation coefficient between the proportion of penicillin resistance for each pair of serotypes in 2009 (red, upper diagonal) and 2012 (blue, bottom diagonal).

Serotype	35B	15BC	23B	16F	23A	15A
35B		0.5224874	0.23571183	0.59731911	0.5732115	0.4130514
15BC	0.6889201		0.64062589	0.38313051	0.11323093	-0.2709142
23B	0.766871	0.8322754		-0.09401268	-0.09526162	-0.3830342
16F	0.4709555	0.5444811	0.301017		0.5954612	0.274986
23A	0.7919866	0.671008	0.5049354	0.4351319		0.6625419
15A	0.711235	0.4483838	0.3250522	0.4276296	0.7822086	

Technical Appendix Table 3. Crude and standardized proportions of all nonvaccine type pneumococci that were penicillin resistant in 2009 and 2012, stratified by state

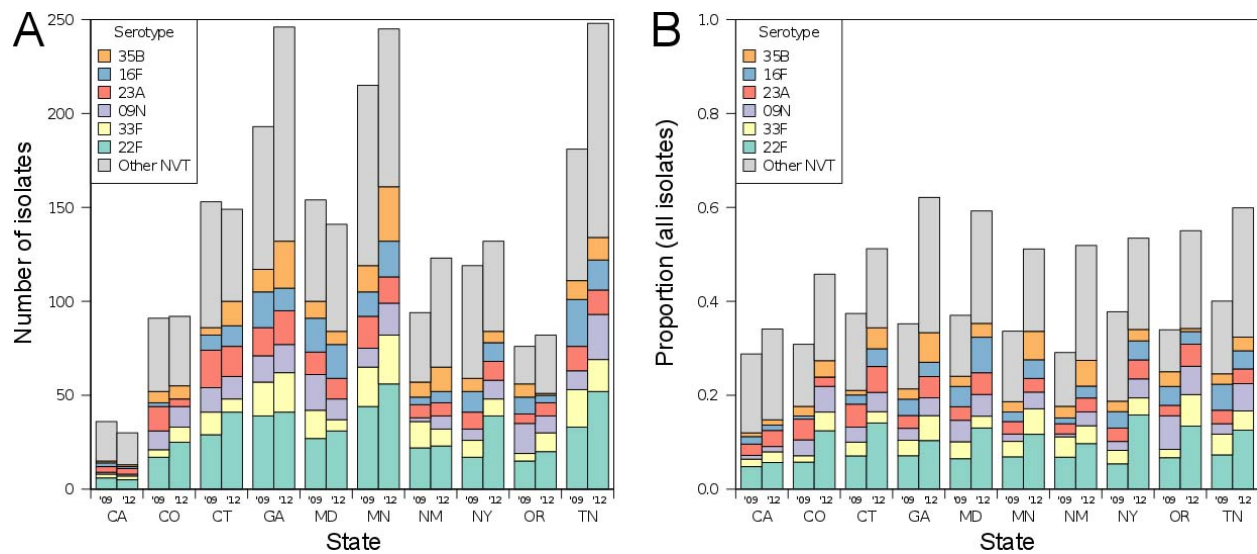
State	2009			2012		
	Crude	Std1	Std2	Crude	Std1	Std2
CA	0.167	0.159	0.238	0.200	0.188	0.278
CO	0.308	0.263	0.238	0.217	0.232	0.210
CT	0.235	0.204	0.238	0.235	0.259	0.269
GA	0.207	0.210	0.221	0.268	0.233	0.234
MD	0.182	0.168	0.222	0.241	0.224	0.244
MN	0.233	0.229	0.216	0.245	0.232	0.240
NM	0.191	0.247	0.164	0.260	0.255	0.239
NY	0.286	0.216	0.290	0.205	0.240	0.196
OR	0.118	0.150	0.188	0.122	0.171	0.186
TN	0.193	0.258	0.165	0.202	0.252	0.176
Regression		0.445	0.463		0.367	0.634
95% CI		(-0.083-0.972)	(-0.013-0.939)		(-0.025-0.758)	(0.14-1.128)

Std1 refers to standardization removing the effects of geographic variation in serotype distribution, while Std2 refers to standardization removing the effects of serotype-specific differences in penicillin resistance levels within individual states. CI refers to confidence interval.

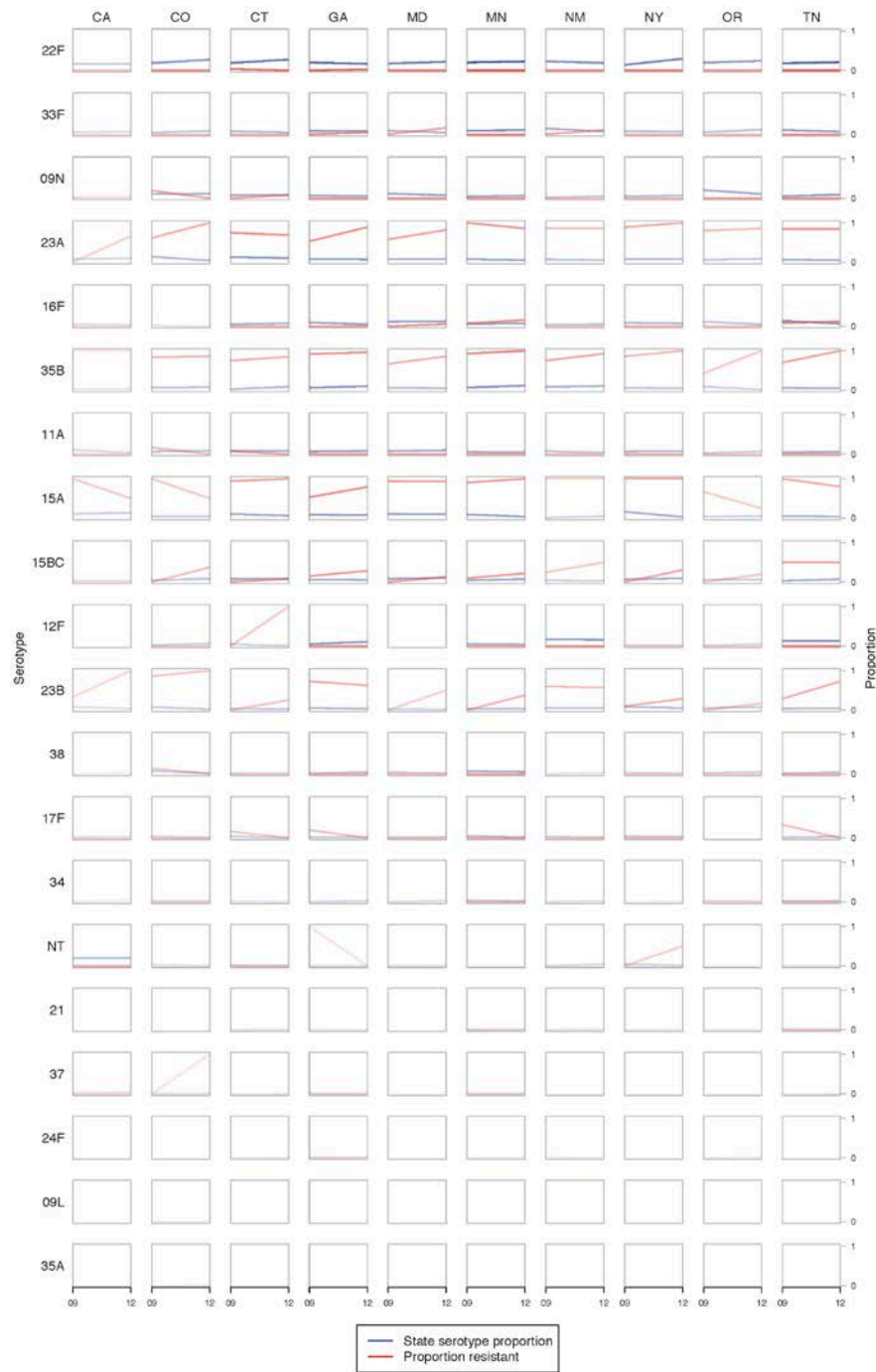
Technical Appendix Table 4. Changes in penicillin resistance among nonvaccine type pneumococcal strains over time by site

State	Proportion 2009	Proportion 2012	Logistic growth rate
NM	0.191 (7)	0.260 (2)	0.132 (-0.083, 0.354)
MD	0.182 (8)	0.241 (4)	0.119 (-0.068, 0.309)
GA	0.207 (5)	0.268 (1)	0.113 (-0.035, 0.264)
CA	0.167 (9)	0.200 (9)	0.074 (-0.351, 0.5)
MN	0.233 (4)	0.245 (3)	0.023 (-0.12, 0.167)
TN	0.193 (6)	0.202 (8)	0.017 (-0.142, 0.18)
OR	0.118 (10)	0.122 (10)	0.011 (-0.311, 0.338)
CT	0.235 (3)	0.235 (5)	-0.001 (-0.179, 0.177)
NY	0.286 (2)	0.205 (7)	-0.147 (-0.343, 0.045)
CO	0.308 (1)	0.217 (6)	-0.157 (-0.382, 0.064)

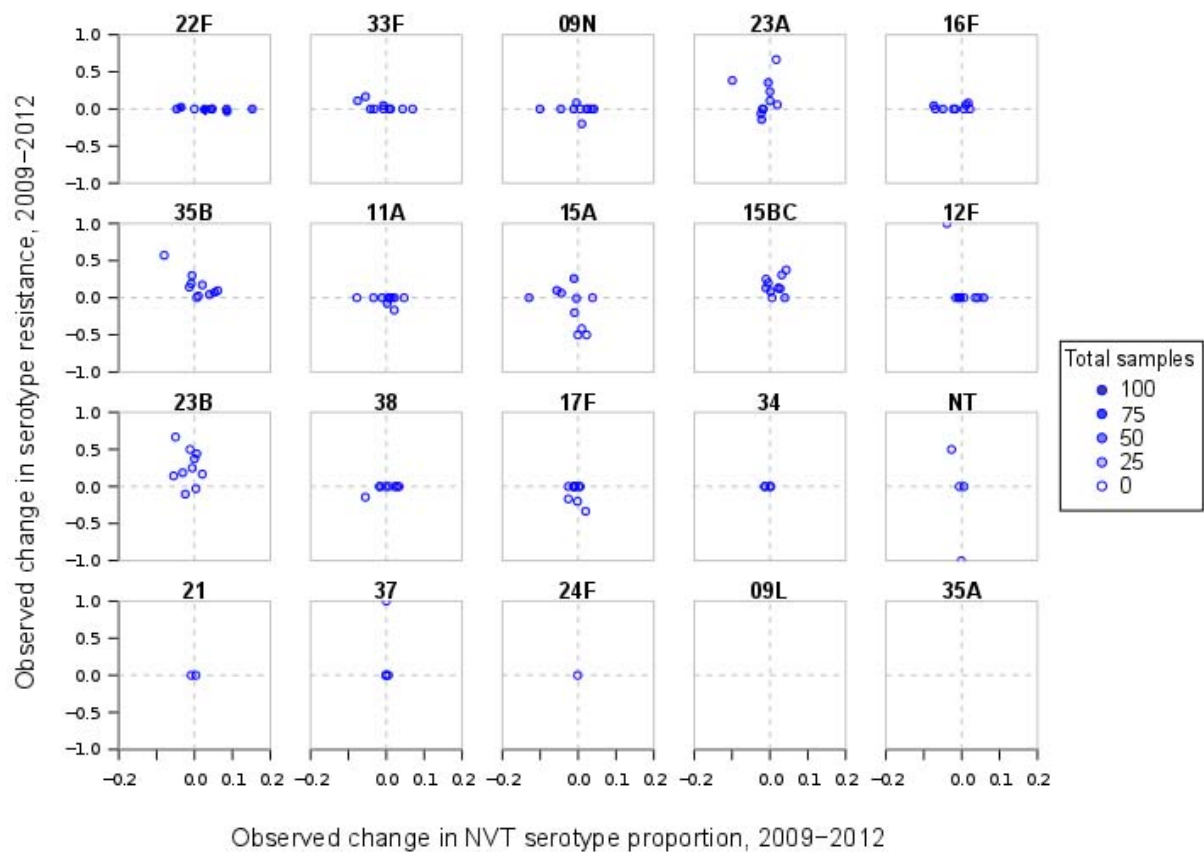
The ranks of each state in both 2009 and 2012 are indicated in parentheses, with the highest proportion of penicillin resistance having a rank of 1. Confidence intervals for the growth rates are shown in parentheses. The states are ordered starting from those with the highest growth rate to the least.



Technical Appendix Figure 1. Sampled NVTs by state and year. A) Total number of NVT isolates. B) NVTs as a proportion of the total sample. The six serotypes shown represent the most common types in the dataset.



Technical Appendix Figure 2. Changes in each NVT serotype as a proportion of the total NVT population (blue) and in the proportion of penicillin resistance (red line) segregated by serotype and by state between 2009 and 2012. The weight of the line colors represent the number of isolates collected, with darker colors representing a greater number of samples.



Technical Appendix Figure 3. Change in serotype proportion against change in penicillin resistance, 2009–2012. Each panel shows a single serotype, and plots for each state shows the change in proportion of NVTs represented by that serotype, and the change in prevalence of penicillin resistance between 2009 and 2012. Points are shaded to represent the combined number of samples in 2009 and 2012 for each state.